# Human IL-6 Super Sensitivity ELISA Kit

Instructions for Use | Cat. No.: AEHSS0001

## 1. Content

| Component | CAT | Volume |
|---|---|---|
| CP (Coated Plate) | EHSS0001CP | 96 well |
| S (Standard) | EHSS0001S,S1~S7,S0 | 9 vial |
| DA (Detect Antibody) | EHSS0001DA | 6 ml/bottle |
| SH (Streptavidin-HRP) | ESHSS03 | 12 ml/bottle |
| AB (Assay Buffer 1×) | EAB01 | 12 ml/bottle |
| TS (TMB Substrate) | ETS01 | 12 ml/bottle |
| SS (Stop Solution) | ESS01 | 12 ml/bottle |
| WB (Wash Buffer 10×) | EWB01 | 50 ml/bottle |
| SF (Sealer Film) | ESF01 | 6 pieces |

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

## 2. Reagent Preparation

### Washing Buffer (1×) Preparation

Pour entire contents (50 ml) of the Washing Buffer Concentrate (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C. ■

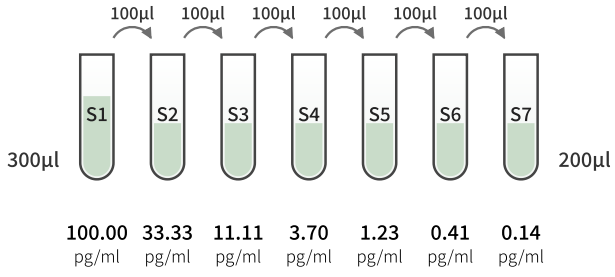
### Standard Curve Preparation

S1 to S7 and S0 is ready to use for serum and plasma.

Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc.

Urine sample use AB (Assay Buffer) prepare standard curve.

The Human IL-6 Standard EHSS0001S 1000 pg/ml 30 µl + 270 µl SPB serves as the high standard (100 pg/ml). Pipette 200 µl of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).



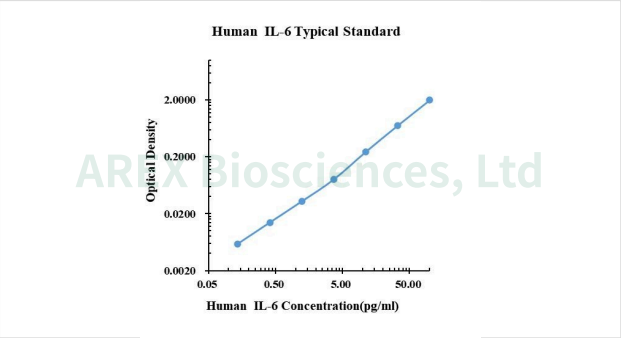

## 3. Assay Procedure

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess CP (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50 µl of AB (Assay Buffer) to each well.
4. Add 50 µl of Standard or sample per well. The volume of the sample added should be consistent with that of the standard. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 µl of DA (Detect Antibody) to each well.
6. Cover with an SF (Sealer Film). Incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker set at 500 rpm.
7. Aspirate each well and wash, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100 µl of SH (Streptavidin-HRP) to each well.
9. Cover with a new SF (Sealer Film). Incubate at room temperature (18 to 25°C) for 30 min on a microplate shaker set at 500 rpm.
10. Repeat aspiration/wash as in step 7.
11. Add 100 µl of TS (TMB Substrate) to each well. Incubate the sample of 10 µl at room temperature for about 20 minutes; incubate the sample of 50 µl at room temperature for about 8 minutes.
12. Add 100 µl of SS (Stop Solution) to each well.
13. Determine the optical density within 30 minutes, using microplate reader set to 450 nm corrected with 570 nm or 630 nm.

## 4. Typical Data

For every test, a standard curve must be established for each microplate.




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| (pg/ml) | O.D. | Average | Corrected |
|---|---|---|---|
| 0.00 | 0.0565 / 0.0573 | 0.0569 | - |
| 0.14 | 0.0621 / 0.0635 | 0.0628 | 0.0059 |
| 0.41 | 0.0701 / 0.0717 | 0.0709 | 0.0140 |
| 1.23 | 0.0919 / 0.0878 | 0.0899 | 0.0330 |
| 3.70 | 0.1406 / 0.1335 | 0.1371 | 0.0802 |
| 11.11 | 0.3042 / 0.2996 | 0.3019 | 0.2450 |
| 33.33 | 0.7676 / 0.7670 | 0.7673 | 0.7104 |
| 100.00 | 2.0951 / 1.9914 | 2.0433 | 1.9864 |

## 5. Sensitivity

The minimum detectable dose (MDD) of human IL-6 with a 10 µl sample is typically less than 0.33 pg/ml ;with a 50 µl sample is typically less than 0.11 pg/ml; The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

## 6. Precision

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested six times on one plate to assess intra-assay precision.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample Number | S1 | S2 | S3 | S1 | S2 | S3 |
| | 22 | 22 | 22 | 6 | 6 | 6 |
| Average(pg/ml) | 1.6 | 10.1 | 31.0 | 1.7 | 9.9 | 29.9 |
| Standard Deviation | 0.1 | 0.3 | 0.8 | 0.1 | 0.4 | 1.1 |
| Coefficient of Variation(%) | 6.8 | 2.7 | 2.5 | 6.7 | 3.8 | 3.6 |

## 7. Recovery

The spike recovery was evaluated by spiking 3 levels of human IL-6 into healthy human serum sample.

The un-spiked serum was used as blank in this experiment. The recovery ranged from 84% to 114% with an overall mean recovery of 99%.

## 8. Linearity

To assess the linearity of the assay, five samples were spiked with high concentration of IL-6 in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 92% to 105% with an overall mean recovery of 98%.

## 9. Sample Values

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of IL-6 in this assay. No medical histories were available for the donors.

| Sample Matrix | Sample Evaluated | Range (pg/ml) | Detectable % | Mean of Detectable (pg/ml) |
|---|---|---|---|---|
| Serum | 30 | 2.45-53.36 | 100 | 23.94 |

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.